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INFLUENCE OF SOAKING DURATION AND PARTICLE SIZE ON ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL CHARACTERISTICS OF *Uncaria Gambir* Roxb ETHANOLIC LEAF EXTRACT TEA

PENGARUH DURASI PERENDAMAN DAN UKURAN PARTIKEL TERHADAP AKTIVITAS ANTIOKSIDAN DAN KARAKTERISTIK FITOKIMIA TEH EKSTRAK DAUN *Uncaria Gambir* Roxb DALAM ETANOL

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Abstract

Environmental pollution and modern lifestyles increase human exposure to free radicals, which contribute to various degenerative diseases. Antioxidants play a crucial role in neutralizing free radicals and preventing oxidative damage. *Uncaria gambir* Roxb., a tropical plant widely used in Indonesia, is rich in polyphenolic compounds, particularly catechins, with strong antioxidant potential. This study aimed to evaluate the antioxidant activity of ethanolic extracts of *Uncaria gambir* leaves through an integrated approach involving in silico prediction, extraction optimization, phytochemical screening, and in vitro antioxidant testing. Bioactive compounds were first screened using PASS Online to predict antioxidant and free radical scavenging activities. Ethanolic maceration was conducted using different particle sizes and maceration times, followed by yield determination. Phytochemical screening was performed to identify major secondary metabolites, and antioxidant activity was assessed using the DPPH radical-scavenging assay. In silico analysis indicated that most polyphenolic compounds exhibited high antioxidant potential. The highest extraction yield was obtained using a 60-mesh particle size and a 6-hour maceration period. Phytochemical screening confirmed a strong presence of phenolic compounds, and the DPPH assay showed a dose-dependent antioxidant response with a low IC_{50} value, indicating strong antioxidant activity. These findings demonstrate that optimized ethanolic extracts of *Uncaria gambir* leaves have significant potential as natural antioxidant sources.

Keywords: *Uncaria gambir* Roxb, *antioxidant activity, PASS Online, DPPH assay, phenolic compounds*

Abstrak

Pencemaran lingkungan dan gaya hidup modern meningkatkan paparan manusia terhadap radikal bebas yang berkontribusi pada berbagai penyakit degeneratif. Antioksidan memainkan peran penting dalam menetralkan radikal bebas dan mencegah kerusakan oksidatif. *Uncaria gambir* Roxb., tanaman tropis yang banyak digunakan di Indonesia, dikenal kaya akan senyawa polifenolik, khususnya katekin, dengan potensi antioksidan yang kuat. Studi ini bertujuan untuk mengevaluasi aktivitas antioksidan ekstrak etanol daun *Uncaria gambir* melalui pendekatan terintegrasi yang melibatkan prediksi in silico, optimasi ekstraksi, skrining fitokimia, dan pengujian antioksidan in vitro. Senyawa bioaktif pertama kali disaring menggunakan PASS Online untuk memprediksi aktivitas antioksidan dan penangkapan radikal bebas. Maserasi etanol dilakukan dengan ukuran partikel dan waktu maserasi yang berbeda, kemudian diikuti dengan penentuan hasil. Skrining fitokimia dilakukan untuk

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mengidentifikasi metabolit sekunder utama, dan aktivitas antioksidan dinilai menggunakan uji penangkapan radikal DPPH. Analisis in silico menunjukkan bahwa sebagian besar senyawa polifenolik memiliki probabilitas antioksidan yang tinggi. Hasil ekstraksi tertinggi diperoleh dengan menggunakan ukuran partikel 60 mesh dan periode maserasi 6 jam. Pemeriksaan fitokimia mengonfirmasi adanya senyawa fenolik yang kuat, dan uji DPPH menunjukkan respons antioksidan yang bergantung pada dosis dengan nilai IC_{50} yang rendah, sehingga menunjukkan aktivitas antioksidan yang kuat. Temuan ini menunjukkan bahwa ekstrak etanol daun *Uncaria gambir* yang dioptimalkan memiliki potensi yang signifikan sebagai sumber antioksidan alami.

Kata kunci: *Uncaria gambir* Roxb, aktivitas antioksidan, PASS Online, uji DPPH, senyawa fenolik

INTRODUCTION

Pollution and modern environmental conditions increase human vulnerability to free radical exposure originating from cigarette smoke, excessive ultraviolet radiation, air pollution, suboptimal nutritional intake, and prolonged stress, which can significantly trigger various degenerative diseases such as cancer, cardiovascular disorders, Alzheimer's disease, Parkinson's disease, and premature aging; however, these detrimental effects can be mitigated by antioxidants (Nanda Pratama & Busman, 2020).

Antioxidants act as essential protective agents in the body by donating electrons to neutralize free radicals and prevent damage to vital biological components, making adequate antioxidant intake through a balanced diet and healthy lifestyle crucial for maintaining overall health (Aditya & Ariyanti, 2016). Antioxidant compounds are widely found in fruits, vegetables, green tea, and herbal teas, which have gained increasing popularity due to the availability of raw materials and their diverse health benefits (Iskandar & Ramdhan, 2020).

Uncaria gambir Roxb (UGR) is a tropical plant traditionally used across various regions of Indonesia and Asia as an antidiarrheal and astringent agent and processed into herbal tea with medicinal value; in West Kalimantan, in addition to its sap, gambir leaves are highly valued for their catechin content, which exhibits strong antioxidant activity (Aditya & Ariyanti, 2016; Iskandar & Ramdhan, 2020). The antioxidant potential of UGR can be predicted using in silico computer-based approaches such as PASS Online, molecular docking, and computer-aided drug design (CADD) (Iskandar *et al*., 2022; Uspenskaya *et al*., 2023; Mohanasundaram *et al*., 2024; Chang *et al*., 2023).

Based on literature studies, commonly used extraction methods include maceration, Soxhlet extraction, reflux, and percolation using ethanol as the solvent, which has been shown to produce higher extraction yields and effectively extract antioxidant compounds compared to other solvents (Maryam *et al*., 2023; Maulida *et al*., 2024; Purwanto *et al*., 2024). Antioxidant activity can be evaluated using DPPH radical scavenging, Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP), and Ferric Reducing Antioxidant Power (FRAP) assays, while phytochemical screening focuses on flavonoids, phenolics, terpenoids, saponins, and alkaloids (Iskandar *et al*., 2022; Sadowska-Bartosz & Bartosz, 2022; Iskandar & Warsidah, 2020). Therefore, this study is important to confirm the presence and strength of antioxidant compounds in ethanolic herbal tea extracts of UGR leaves and to further develop the potential utilization of underexplored local plants as natural antioxidant sources

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RESEARCH METHODS

In Silico PASS Online

In silico screening of bioactive compounds from *Uncaria gambir* Roxb (UGR) was conducted using the PASS Online approach. The initial step involved accessing the KNApSAcK family database via http://www.knapsackfamily.com/KNApSAcK/. Bioactive compounds were searched using the keyword "*Uncaria gambir* plant." All retrieved compound data were downloaded and organized using Microsoft Excel for data management. The identified bioactive compounds of UGR were then unified by checking the canonical SMILES of each compound using the PASS Online database at https://www.way2drug.com/. The verified canonical SMILES were subsequently used to predict the biological activity of the compounds, particularly their potential antioxidant activity contained in the UGR plant (Iskandar *et al*., 2022).

Extraction of UGR Leaves

The extraction process of UGR leaves was initiated by weighing 5 g of leaf samples. The leaves were then reduced in size according to the applied treatments. In the first treatment, the leaves were ground in a blender and sieved through a 60-mesh screen, while in the second treatment, the leaves were chopped into pieces approximately 0.25 cm in size. The prepared samples were placed in containers and submerged in ethanol. The soaking process was carried out for 2, 4, and 6 hours. After soaking, the extract was pressed with gauze cloth in a mechanical press and then filtered through filter paper to obtain a filtrate free of impurities. The filtrate was concentrated using an evaporator and further evaporated in a water bath at 50°C to obtain a viscous extract. The resulting extract was weighed to calculate the extraction yield and stored in labeled bottles as ethanolic UGR extract. Large-scale extraction was subsequently performed following the treatment that produced the highest yield. The percentage yield was calculated using the following formula:

$$\text{Percentage yield} = (\text{mass of dry extract} / 50\ \text{g}) \times 100\%$$ (Iskandar *et al*., 2022).

Phytochemical Screening

Alkaloid testing was conducted by preparing 2 ml of sample solution, which was heated on a porcelain dish until a residue was obtained. The residue was then dissolved in 5 mL of 2 N HCl and divided into four test tubes. The first tube served as a blank with the addition of dilute acid, while the second, third, and fourth tubes were each added with three drops of Dragendorff, Mayer, and Wagner reagents, respectively. The formation of a red precipitate in the Dragendorff tube, a yellow precipitate in the Mayer tube, and a brown or reddish precipitate in the Wagner tube indicated the presence of alkaloids. Phenolic compounds were tested by dividing the sample solution into two test tubes, where tube A served as a blank and tube B was reacted with 10% $FeCl_3$ solution. A color change to bluish-black indicated the presence of phenolic compounds. Flavonoid testing was performed by adding 2 mL of the sample solution to a test tube, followed by adding 3 drops of 10% NaOH solution. The yellow color indicated the presence of flavonoids. Terpenoid testing was carried out by evaporating 2 mL of the sample solution on a porcelain dish until dry, then adding

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Ciulei reagent consisting of chloroform, acetic anhydride, and concentrated sulfuric acid. The appearance of a brown color indicated the presence of terpenoids. Saponin testing was performed by adding 2 mL of the sample solution to a test tube, followed by adding 10 mL of distilled water and homogenizing. The formation of stable foam indicated the presence of saponins (Iskandar & Ramdhan, 2020).

Determination of Antioxidant Activity Using the DPPH Method

Antioxidant activity was determined using the DPPH radical scavenging method. A stock solution was prepared by dissolving 10 ml of UGR extract in 50 ml of ethanol, followed by serial dilution to obtain the desired concentrations. The DPPH stock solution was prepared by dissolving 5 mg of DPPH powder in 100 mL of analytical-grade methanol, then homogenized with a vortex mixer. For the assay, 2 mL of each sample solution was mixed with 2 mL of DPPH solution and incubated for 30 minutes at 27°C until a color change occurred. All measurements were performed in duplicate. Absorbance was measured using a spectrophotometer at 517 nm. The IC_{50} value was calculated to assess antioxidant strength; an IC_{50} below 50 µg/mL indicates high antioxidant activity. Sample dilution was performed based on standard dilution calculations (Aditya & Ariyanti, 2016).

The study employed a factorial Completely Randomized Design (CRD) with two treatment factors, namely maceration time (2, 4, and 6 hours) as the first factor and particle size (60 mesh and chopped) as the second factor. All treatments were carried out in three replications (triplicate), resulting in a total of 18 experimental units. Observations were conducted through direct examination and analysis of antioxidant activity results using the DPPH method. The data obtained included the results of phytochemical screening and absorbance values at specific wavelengths from the ethanolic extract of *Uncaria gambir* Roxb. leaves with variations in maceration time and particle size. To determine significant differences among treatment means, the experimental data were analyzed using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

1. Results

Table 1. In silico PASS Online Results

No	Compound	Antioxidant (Pa)	Free Radical Scavenger (Pa)
1	(+)-Catechin	0.81	0.842
2	(–)-Epicatechin	0.81	0.842
3	(+)-Epicatechin	0.81	0.842
4	Mitraphylline	–	–
5	Roxburghine B	–	–
6	Catechol	0.501	0.413
7	Gallic acid	0.52	–
8	Cinchonain Ia	0.856	0.716
9	Gambiriin C	0.803	0.798
10	Gambiriin A1	0.647	0.792
11	Gambiriin A3	0.802	0.808

No	Compound	Antioxidant (Pa)	Free Radical Scavenger (Pa)
12	Gambiriin B1	0.593	0.665
13	Gambiriin B3	0.727	0.742
14	Procyanidin B3	0.803	0.798
15	Procyanidin B1	0.803	0.798
16	Uncarine B	–	–
17	Gambiriin A2	0.647	0.792
18	Gambiriin B2	0.703	0.815

Table 2. Yield Percentage Data of UGR Maceration

No	Sample Code	Final Weight (g) Rep. 1	Rep. 2	Rep. 3	Mean (g)	Yield (%)
1	U1W1	0.9	0.8	0.8	0.8	16
2	U2W1	0.6	0.7	0.8	0.7	14
3	U1W2	1.2	1.1	1.1	1.1	22
4	U2W2	1.1	0.9	0.9	0.9	18
5	U1W3	1.4	1.6	1.6	1.5	30
6	U2W3	1.3	1.2	1.2	1.2	24

Notes: U1W1 represents a 60 mesh particle size with a 2-hour maceration period, U2W1 indicates a chopped particle size with a 2-hour maceration period, U1W2 refers to a 60 mesh particle size with a 4-hour maceration period, U2W2 denotes a chopped particle size with a 4-hour maceration period, U1W3 corresponds to a 60 mesh particle size with a 6-hour maceration period, and U2W3 indicates a chopped particle size with a 6-hour maceration period.

Table 3. Phytochemical Screening Results

Chemical Compound	Reagent	Result
Alkaloids	Wagner	–
	Mayer	–
Flavonoids	NaOH 10%	–
Phenolics	$FeCl_3$ 10%	++
Terpenes	Ciulei	+
Saponins	Distilled water	+

Notes: "++" indicates that the result was very clearly observed, "+" indicates that it was clearly observed, and "–" indicates that it was not observed.

Table 4 Results of the DPPH assay of the ethanol extract of *Uncaria gambir* (UGR)

Concentration (ppm)	Blank	Absorbance (Duplicate)	% Inhibition	Mean (%)	IC_{50}
5	0.791	0.455	42.478	42.225	7.045
		0.459	41.972		
10		0.353	55.373	57.901	
		0.313	60.43		
15		0.205	74.083	73.957	
		0.207	73.831		
20		0.182	76.991	81.479	
		0.111	85.967		
25		0.047	94.058	91.34	
		0.09	88.622		

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2. Discussion

The in silico PASS Online analysis revealed that the majority of bioactive compounds identified in *Uncaria gambir* Roxb. leaves, particularly catechins, epicatechins, procyanidins, cinchonain Ia, and gambiriin derivatives, exhibited high probabilities (Pa ≥ 0.7) of being antioxidants and free radical scavengers. These findings indicate strong theoretical potential for antioxidant activity and confirm that polyphenolic constituents are the primary contributors to UGR's bioactivity. Recent studies have demonstrated that flavan-3-ols such as catechin and epicatechin possess strong electron-donating capacity and effectively neutralize reactive oxygen species through hydrogen atom transfer and single-electron transfer mechanisms (Mahfudh *et al*., 2024; Wang *et al*., 2023). The absence of predicted antioxidant activity for alkaloids such as mitraphylline and uncarine B further supports the dominance of phenolic compounds in determining the antioxidant profile of Uncaria gambir.

The extraction yield results showed that both maceration time and particle size significantly influenced extract recovery, with the highest yield obtained using a 60-mesh particle size and a 6-hour maceration period. This trend is consistent with mass transfer theory, in which smaller particle size increases surface area, and longer maceration time enhances solvent penetration and solute diffusion. Similar observations have been reported in recent extraction optimization studies, which showed that prolonged maceration and reduced particle size significantly improve phenolic extraction efficiency when ethanol is used as the solvent (Syukri *et al*., 2023; Sun *et al*., 2025). Ethanol has been widely recommended for polyphenol extraction due to its polarity, safety, and ability to solubilize both low- and medium-molecular-weight phenolic compounds (Palos-Hernández *et al*., 2024).

Phytochemical screening confirmed the strong presence of phenolic compounds, while terpenes and saponins were moderately detected, and alkaloids and flavonoids were not observed using qualitative reagents. The strong $FeCl_3$ reaction supports the abundance of phenolic hydroxyl groups, which are responsible for antioxidant activity. The negative result for flavonoids in qualitative testing may be attributed to the structural characteristics of flavan-3-ols, such as catechins, which do not always respond positively to alkaline reagents used in conventional flavonoid assays (Sahraeian, 2024). Recent studies emphasize that qualitative phytochemical tests may underestimate certain polyphenol subclasses, and chromatographic or spectrometric techniques are often required for accurate identification (Lu *et al*., 2025).

The DPPH radical-scavenging assay demonstrated a clear dose-dependent increase in antioxidant activity, with high inhibition percentages at higher concentrations and a low IC_{50} value, indicating the strong antioxidant capacity of the ethanolic UGR extract. This result is consistent with recent reports linking high total phenolic content to strong DPPH scavenging activity in Uncaria gambir and other polyphenol-rich medicinal plants (Hidayati *et al*., 2022; Wardana *et al*., 2023). The strong agreement between in silico predictions, phytochemical screening, extraction yield trends, and in vitro antioxidant activity highlights the reliability of combining computational screening with experimental validation. Overall, this study confirms that optimized ethanolic extraction of *Uncaria gambir* leaves yields phenolic-rich extracts with potent antioxidant activity, supporting their potential application as natural antioxidants in pharmaceutical and functional food formulations.

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REFERENCES

Aditya, M., & Ariyanti, P. R. (2016). Antioxidant activity analysis using the DPPH radical scavenging method. Journal of Applied Pharmaceutical Science, 6(4), 123–130.

Hidayati, D. N., Safitri, E. I., Alviani, D. L., & Putri, M. N. A. (2022). Cytotoxic activity and inhibition of 4T1 breast cancer cell migration from plant leaf extracts. Jurnal Tumbuhan Obat Indonesia, 15(1), 41–47. https://doi.org/10.22435/jtoi.v15i1.5710

Iskandar, D., Widodo, N., Warsito, W., Masruri, M., & Rollando, R. (2022). A review of ethnomedicinal plants used in West Kalimantan. International Journal of Agricultural Sciences, 6(1), 27–41. https://doi.org/10.25077/ijasc.6.1.27-41.2022

Iskandar, D., & Ramdhan, R. (2020). Phytochemical screening and antioxidant activity of medicinal plant extracts. Journal of Herbal Science and Technology, 4(2), 55–63.

Lu, Y., Wang, K., & Hu, L. (2025). Advancements in delivery systems for dietary polyphenols in enhancing health effects: Challenges and opportunities. npj Science of Food, 9, Article 51. https://doi.org/10.1038/s41538-025-00251-9

Mahfudh, M., Pratama, R. A., & Lestari, D. (2024). Polyphenolic compounds as natural antioxidants: Mechanisms and in silico prediction approaches. Food Chemistry Advances, 3, 100312. https://doi.org/10.1016/j.focha.2024.100312

Palos-Hernández, A., García-Villanova, B., & Guerra-Hernández, E. (2024). Latest advances in green extraction of polyphenols from plants and their bioactive compounds. Molecules, 29(6), 1284. https://doi.org/10.3390/molecules29061284

Sahraeian, S. (2024). Recent advances in conjugation methods to improve polyphenol bioavailability: Concepts and applications. Food Chemistry Reviews, 1(2), 100015. https://doi.org/10.1016/j.fchrev.2024.100015

Sun, S., Zhang, Y., Li, M., & Chen, J. (2025). Impact of extraction techniques on phytochemical profiles and bioactivity of natural product extracts: A comprehensive review. Journal of Ethnopharmacology, 334, 117623. https://doi.org/10.1016/j.jep.2024.117623

Syukri, D., Rahman, A., & Putri, N. R. (2023). Optimization of extraction parameters on phenolic yield and antioxidant activity of medicinal plant extracts. Andalas International Journal of Agricultural and Natural Sciences, 4(2), 88–97.

Wang, X., Wang, Y., & Zhang, H. (2023). Mechanisms of polyphenols in scavenging free radicals and regulating oxidative stress. Food & Function, 14(19), 8721–8735. https://doi.org/10.1039/D3FO01844A

Wardana, A. P., Aminah, N. S., Kristanti, A. N., Fahmi, M. Z., Zahrah, H. I., Widiyastuti, W., Ajiz, H. A., Zubaidah, U., Wiratama, P. A., & Takaya, Y. (2023). Nano *Uncaria gambir* as a chemopreventive agent against breast cancer. International Journal of Nanomedicine, 18, 4471–4484. https://doi.org/10.2147/IJN.S403385